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Host-Zooxanthella Interactions in Four Temperate Marine Invertebrate Symbioses: Assessment of Effect of Host Extracts on Symbionts

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Abstract. Photosynthesis and translocation of photosynthetic products from symbiotic zooxanthellae in four species of temperate-latitude invertebrates were investigated *in vivo* and *in vitro*. *In vivo*, zooxanthellae fixed ^{14}C and translocated a substantial proportion of fixed products to host tissues. *In vitro*, the effect of host tissue extracts on isolated zooxanthellae varied. Extracts of the soft coral *Capnella gaboensis*, lysed zooxanthellae after a relatively short exposure. Those of the zoanthid *Zoanthus robustus* and the nudibranch *Pteraeolidia ianthina* had little effect on translocation of organic carbon from zooxanthellae. In contrast, host extract of the scleractinian coral *Plesiastrea versipora* stimulated the release of up to 42% of the total ^{14}C fixed, and the magnitude of release was positively correlated with the protein concentration of the extract. Host extracts had no effect on photosynthetic rates in algal symbionts.

The effect of *P. versipora* extract on isolated zooxanthellae was studied. This extract caused zooxanthellae to divert photosynthetic products from lipid synthesis to the production of neutral compounds, principally glycerol, and these compounds were the predominant form of carbon detected extracellularly after incubating zooxanthellae in this extract. Only organic compounds made during the period of exposure of zooxanthellae to host extract, and not pre-formed photosynthetic products, were translocated. The translocation-inducing activity of host extract was almost completely destroyed by heating (100°C), and a preliminary attempt to fractionate the tissue extract revealed that the active constituent did not pass through dialysis tubing of nominal pore size 10,000 D. These results are discussed in relation to host control of symbiotic partners, and to previous reports of "host-release factors" in other invertebrate symbioses.

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Introduction

Many marine invertebrates belonging to the phyla Mollusca, Platyhelminthes, Cnidaria, and Protozoa contain endosymbiotic dinoflagellates, collectively known as zooxanthellae. In nudibranch molluscs (Rudman, 1981a, 1982) and the majority of cnidarians (Trench, 1979), zooxanthellae are found within vacuoles in host cells derived from the endoderm. Zooxanthellae carry out photosynthesis within the confines of the host cell, and make significant contributions to host cell metabolism by translocation of organic compounds to the host (for review, see Trench, 1979). Muscatine and co-workers estimate that up to 95% of the carbon fixed during photosynthesis is translocated to coral hosts (Muscatine *et al.*, 1983, 1984), and at least some of the translocated carbon is used by the host for respiration and growth (Franzisket, 1970; Johannes, 1974; Kevin and Hudson, 1979; Kempf, 1984). Moreover, the animal provides organic and inorganic nutrients, some of them metabolic waste products, to the algae (Cook, 1971; Muscatine and Porter, 1977).

Most of the studies noted above concern tropical symbioses. In temperate latitudes, many marine symbiotic associations involve zooxanthellae, and a number of studies of these associations show that zooxanthellae contribute to metabolic processes in their hosts and to

calcification rates (Jacques and Pilson, 1980; Jacques *et al.*, 1983; Tytler and Davies, 1986). However, the extent and significance of *in vivo* translocation of photosynthetic products to host tissues in these interactions have rarely been studied.

Little is known about the control of translocation between the partners in symbiosis. Several *in vitro* studies have suggested that the host may contain compounds ("host factors") that cause carbon to be translocated from the alga (Muscatine, 1967; Trench, 1971c), but no chemical entity with this function has been identified, and no mechanism for host factor action has been demonstrated. In some associations, host factors have been reported only in extracts of symbiotic hosts and not in extracts from aposymbiotic individuals (Trench, 1971c). Host factors from some (Muscatine, 1967; Yu and Dietrich, 1977), but not all (Muscatine *et al.*, 1972) symbiotic invertebrates are heat-labile, and cross-reactivity experiments suggest that invertebrates having zooxanthellae have similar host factors. Thus, host extract from the symbiotic clam *Tridacna* stimulates the release of organic carbon from zooxanthellae from the coral, *Pocillopora damicornis*, and the coral extract has a similar effect on clam zooxanthellae (Muscatine, 1967). Host factors may also influence the photosynthetic rate of zooxanthellae (Trench, 1971c; Muscatine *et al.*, 1972), and their alanine uptake (Carroll and Blanquet, 1984b; Blanquet *et al.*, 1988). In summary, the distribution of host factors and their effects among invertebrates having zooxanthellae is obscure.

This study had three objectives: the first was to determine whether *in vivo* translocation in a range of temperate invertebrates occurs. To this end, four relatively abundant marine invertebrates from Latitude 34°S, New South Wales, Australia, having zooxanthellae as symbionts and representing diverse taxa, were chosen for experimentation: the soft coral *Capnella gaboensis*, the stony coral *Plesiastrea versipora*, the zoanthid *Zoanthus robustus*, and the aeolid nudibranch *Pteraeolidia ianthina*. The second objective was to determine whether there was evidence, in these temperate marine invertebrate symbioses, for host factor control of zooxanthellar processes, particularly of photosynthesis, metabolism, and translocation of photosynthetic products. The final objective was to characterize the active components of host extracts affecting zooxanthellar processes, should they be found.

Materials and Methods

Source and maintenance of animals

The invertebrates used in this study were collected from the Sydney region, New South Wales, Australia (Latitude 34° S), between May 1980 and May 1981. The nudibranch *Pteraeolidia ianthina* was collected at Ben Buckler Point at depths of 20 m. *Plesiastrea versipora*, *Capnella gaboensis*, and *Zoanthus robustus* were collected at 5-m depth at Port Jackson. Specimens were placed in seawater in plastic bags, transported to the laboratory, and kept in seawater aquaria illuminated by cool white fluorescent lighting (photoperiod 12 h light/12 h dark; 12 $\mu E \cdot m^{-2} \cdot s^{-1}$) in an air conditioned room (21 ± 2°C). Animals were used in experiments within three weeks of collection.

Isolation of zooxanthellae and preparation of host extracts

Unless otherwise stated, filtered (0.45 μm, Millipore) natural seawater was used in all experiments. Suspensions of zooxanthellae were prepared from each species as follows: the surfaces of small colonies (approximately 100 cm^2) of *P. versipora* were abraded with a stainless steel brush, flooded with seawater, and scraped with a nylon toothbrush into a glass dish. For *C. gaboensis*, several branch tips (up to 50 g wet weight) were macerated using a mortar and pestle. The resulting slurries from each animal were passed through one layer of Miracloth (Calbiochem) and made up to 10 ml total volume with seawater. Whole individuals of *Z. robustus* were split longitudinally with a scalpel, and the gastrodermis, containing the zooxanthellae, was scraped into 10 ml of seawater. The cerata of several individuals of *Pa. ianthina* were excised and homogenized in 5 ml of seawater, using a ground-glass homogenizer. Microscopic observations indicated that these techniques did not disrupt zooxanthellae.

The suspensions of zooxanthellae were centrifuged at 2000 rpm (490 × *g*) for 60 s (M.S.E. benchtop centrifuge). The supernatant ("host extract") was poured off. The zooxanthellae were then washed three times by resuspension in 10 ml of seawater followed by re-centrifugation. The pH of the host extract (pH = 7.5–7.9) was adjusted to that of seawater (pH = 8.1) with 0.1 *N* NaOH. The extract was stored at 5°C for not more than 20 min until used. The protein concentration of each extract was determined at the end of each experiment (Lowry *et al.*, 1951), from samples frozen (0°C) following extract preparation.

Electron microscopy

Isolated zooxanthellae and small pieces of intact tissue were fixed for 3–4 h in 3% glutaraldehyde in sodium cacodylate buffer (pH 7.3) and then washed for 10 min in each of three changes of 0.1 *M* sodium cacodylate buffer. Fixed sections of coral tissues were decalcified in a solution of 10% sucrose containing 3% EDTA (sodium ethylenediaminetetraacetic acid; Borowitzka and Vesk,

1978). Specimens were post-fixed for 1 h in 1.0% osmium tetroxide in sodium cacodylate buffer, dehydrated through an acetone series, and embedded in Spurr's (1969) resin. Sections were cut, stained with lead acetate and uranyl acetate, and examined with a Philips-300 transmission electron microscope.

Measurement of release of ^{14}C from zooxanthellae

In the intact association. Whole zoanthids, or 2 cm branch tips of *C. gaboensis*, were incubated in seawater containing 15 $\mu Ci^{14}C \cdot ml^{-1}$ (as $NaH^{14}CO_3$; Amersham) at 25°C under fluorescent light (78–80 $\mu E \cdot m^{-2} \cdot s^{-1}$). After 1 h, the zooxanthellae and host tissues were separated (see above), and the radioactivity of each fraction was measured using liquid scintillation counting. Supernatants were made up to a known volume, while zooxanthellae were resuspended in 20 ml of distilled water. Three 100-μl subsamples were taken from the supernatants and three 50-μl subsamples from the resuspended zooxanthellae. Each subsample was acidified with 100 μl of 0.1 *M* HCl and left in a fume hood for 4 h. Scintillation fluid (10 ml) was added to each subsample and the vial shaken to ensure mixing. The scintillation cocktail contained 0.2 g POPOP, 3.0 g PPO, and 0.5 l Teric-10 dissolved in toluene (1 l). *In vivo* experiments with *P. versipora* were not undertaken because the host tissues could not be completely extracted, nor all the zooxanthellae removed from the calcareous skeleton.

In vitro. Freshly isolated zooxanthellae ($10^6 \cdot ml^{-1}$) were incubated in 2 ml of host extract or seawater in 20 ml glass scintillation vials on a linear shaker (Grant SS40, 100 strokes $\cdot min^{-1}$) under fluorescent light (78–80 $\mu E \cdot m^{-2} \cdot s^{-1}$). At the beginning of each experiment, 10 $\mu Ci \cdot ml^{-1}$ $NaH^{14}CO_3$ was added and the vials shaken to ensure complete mixing. Triplicate 50-μl samples were taken immediately following mixing for the determination of specific activity. Each 50 μl was added to 10 ml of scintillation fluid (see above) made basic by the addition of 0.1 ml of 1.0 *M* NaOH. The radioactivity of each sample was counted for 5 min in a Packard Tri-Carb scintillation counter. Counts were corrected for quenching and background radioactivity and expressed as mg C using calculated specific activities. Calculation of specific activities was based on the total inorganic carbon content of seawater (2.52×10^{-2} mg $C \cdot ml^{-1}$, Skirrow, 1975).

Following incubation, triplicate 50-μl samples were removed from each treatment and filtered under vacuum (0.45 μm, Millipore). The filters were washed three times under vacuum with 0.65 ml of seawater to give a total filtrate volume of 2.0 ml. Three 100-μl subsamples from each filtrate were acidified with 100 μl 0.1 *M* HCl before adding 10 ml of scintillation fluid. The filters supporting zooxanthellae were dissolved in 1.0 ml 2'-methoxyethanol before adding 100 μl 0.1 *M* HCl and 10 ml of scintillation fluid. The samples were counted as described above.

The percentage of photosynthetic products released from the zooxanthellae during incubation was calculated from the ratio of the filtrate activity (^{14}C released by the algae) to the total activity (filtrate plus filter, total fixed ^{14}C).

Measurement of photosynthetic rates

Photosynthetic rates in zooxanthellae in each treatment were determined from the total ^{14}C fixed during the experiment (*i.e.*, the radioactivity of filter and filtrate combined), the specific activity and the total chlorophyll content (determined for each vial at the end of each experiment). Rates were calculated as ^{14}C-carbon fixed per mg total chlorophyll per hour. Total chlorophyll was measured using the methods of Jeffrey and Humphrey (1975).

Identification of labeled compounds

Zooxanthellae were incubated in 2 ml of seawater or host extract with 25 $\mu Ci \cdot ml^{-1}$ $NaH^{14}CO_3$. After 1 h, the zooxanthellae were removed from suspension by centrifugation, and resuspended in 3 ml of distilled water. Supernatants and suspensions of zooxanthellae were extracted by the methanol/formic acid/chloroform procedure of Barnes and Crossland (1978). The resulting chloroform phase (lipid fraction) was made up to 20 ml with chloroform, while the methanol/formic acid phase was dried at 65°C, and redissolved in 20 ml of distilled water. The radioactivity of each fraction was measured by scintillation counting as described above.

The methanol/formic acid extract was separated into neutral, acidic, and basic fractions by ion-exchange chromatography (on Sephadex SP and QAE) using Redgwell's (1980) method. The eluted fractions from the ion-exchange columns were dried and resuspended in 0.5 ml ethanol (neutral compounds and organic acids) or pyridine (amino acids and phosphate esters). Compounds in each ion-exchange fraction were separated by thin-layer chromatography on cellulose plates (Machery-Nagel Cel 300, 10 cm × 10 cm). Plates were spotted with 60 μl of sample and 20 μg of each of a set of nonradioactive standards. Basic fractions (amino acids) were chromatographed twice in the same direction in pyridine:dioxane: NH_4OH (25%):water (2:2:1:1, v/v; G.O. Kirst pers. com.). The acidic (organic acids) and neutral fractions were chromatographed twice in the same direction in EDTA:NH_4OH:water:n-propanol:isopropanol:n-butanol:isobutyric acid (0.25:20:190:70:15:15:500, w/v/v/v/v/v/v; Feige *et al.*, 1969). After drying, the compounds on each plate were visualized using a range of chemically

sensitive sprays. Ninhydrin spray reagent (Gelman catalog No. 72818) was used to detect amino acids, aniline/xylose was used for organic acids (Smith, 1960), and silver nitrate was used for monosaccharides, carbohydrates, and phosphate esters (Smith, 1960). Plates were also exposed to X-ray film (Kodak X-Omat S) for 8 weeks; the film was then developed to determine the distribution of radioactively labeled compounds. To measure the amount of ^{14}C incorporated into a given compound, the cellulose powder from each spot was scraped off and the radioactivity determined by liquid scintillation counting, as described above.

Effect of host extracts on zooxanthellae from other invertebrates

Experiments were undertaken to assess the effects of host extracts on zooxanthellae from other animals used in this study. *C. gaboensis* zooxanthellae and host extract were not included. Extracts and zooxanthellae were prepared as described above. At the beginning of the experiment, zooxanthellae from each host were resuspended in extracts of *P. versipora, Pa. ianthina, Z. robustus,* or seawater. Incubation conditions with ^{14}C, and subsequent analyses, were as described above for *in vitro* studies.

Effect of P. versipora *host extract on pre-formed products of photosynthesis*

To obtain additional information on the biochemical effects of host extracts on zooxanthellae, experiments were performed to determine if previously formed photosynthetic products are subsequently released during incubation in host extract. Zooxanthellae were incubated for one hour in seawater containing 5 $\mu Ci \cdot ml^{-1}$ $NaH^{14}CO_3$ then washed three times by centrifugation and resuspension. The zooxanthellae were then incubated for 1 h in host extract, at which time release of the "pre-formed" labeled products (formed prior to exposure to host extract) was determined. In a parallel experiment, zooxanthellae were first incubated for 1 h in seawater without ^{14}C, washed as above, then incubated in host extract, this time with $NaH^{14}CO_3$. After 1 h of incubation, release of labeled products (formed during incubation in host extract) was determined.

Effect of boiling and dialysis on P. versipora *host extracts*

To determine the heat-sensitivity of *P. versipora* extract, a freshly prepared sample was incubated in a water bath (100°C) for 10 min then cooled to room temperature. The ability of heated extract to stimulate the release of carbon from zooxanthellae was compared with that of unheated host extract.

To determine the effect of dialysis on host extract activity, a freshly prepared sample was first centrifuged for 3 min at 12,000 rpm (27,000 × *g;* 4°C; M.S.E. High Speed 18). The supernatant was filtered (0.45 μm; Millipore HA), then dialysed (Selby's, type 20, nominal pore size 10,000 D) for 6 h with rapid stirring at 4°C (two changes of 1 l buffered seawater). The ability of this treated extract to stimulate release of ^{14}C products from zooxanthellae was compared with that of undialysed extract stored at 4°C for 6 h. Dialysis tubing was boiled in deionized water for 2 h, and rinsed extensively in seawater prior to use.

Results

Photosynthesis and the release of organic ^{14}C from zooxanthellae

In the intact association (see Table I). Zooxanthellae in *C. gaboensis, Z. robustus,* and *Pa. ianthina* photosynthesized at rates ranging from 0.109 to 0.221 mg C · mg chlorophyll$^{-1} \cdot h^{-1}$. Dark fixation rates varied from 2.7% to 6.4% of rates in the light. After one hour, a significant portion (up to 47%) of the fixed ^{14}C-carbon was found associated with host tissues. The percentage of total fixed ^{14}C-carbon detected in tissues of *Z. robustus* during July was significantly lower than at other times of the year. As noted previously, *in vivo* experiments with *P. versipora* were not conducted due to problems in complete extraction of host tissues from the calcarious skeleton.

In vitro *(see Tables IIa, IIb and III).* Zooxanthellae isolated from all the hosts photosynthesized in seawater at rates that ranged between 0.503 and 0.886 mg C · mg chlorophyll$^{-1} \cdot h^{-1}$ (Table IIa). Dark fixation rates in seawater were 2.0–4.0% of those in light. Photosynthetic rates for zooxanthellae in host extracts were not significantly different from those in seawater ($P < 0.05$), except for *C. gaboensis.* In this case, rates in host extract were less than 1% of those in seawater, suggesting damage to algal cells or inhibition of photosynthesis. Subsequent microscopic examination revealed that all cells were lysed after incubation for one hour in this host extract (Fig. 1a). In view of this observation, zooxanthellae incubated in extracts of the other hosts were also examined microscopically (Fig. 1b, *P. versipora*), but they showed no signs of damage and were indistinguishable from those fixed in the host.

Zooxanthellae from all hosts retained approximately 95% of the organic ^{14}C fixed during incubation for 1 h in seawater. In host extracts, zooxanthellae released differing proportions of the organic ^{14}C fixed during 1 h of incubation (Table IIb). For *P. versipora,* the percentage of fixed carbon found outside zooxanthellae in host extract was significantly higher than that released in seawater ($P < 0.01$) and ranged from 10.9 to 42% of the total

Table I

Photosynthetic rates in, and percentage translocation of fixed ^{14}C-products from, zooxanthellae in vivo

Host	Experiment number[1]	% Fixed ^{14}C-products translocated[2]	Photosynthetic rate (light)[2,3]	Dark fixation rate[2,3]
Zoanthus robustus	1	35.2 ± 11.9	0.221 ± 0.092	0.006 ± 0.012
	2	42.2 ± 5.2	n.d.[4]	n.d.
	3	11.8 ± 6.2	0.126 ± 0.022	0.008 ± 0.009
Pteraeolidia ianthina[5]	1	23.8 ± 6.2	0.179 ± 0.080	0.007 ± 0.004
	2	47.5 ± 14.4	0.166 ± 0.026	0.006 ± 0.003
	3	25.1 ± 4.0	0.109 ± 0.027	0.007 ± 0.017
Capnella gaboensis	1	18.1 ± 3.5	0.185 ± 0.074	n.d.
	2	19.1 ± 3.1	0.135 ± 0.036	n.d.

[1] Experiment numbers represent investigations done in March (1), May (2), and July (3) 1981.
[2] Mean ± 95% confidence interval; n = 3 for all experiments.
[3] mg C · mg chlorophyll^{-1} · h^{-1}.
[4] n.d.—not determined.
[5] Data for *Pa. ianthina* from Hoegh-Guldberg and Hinde, 1986.

^{14}C fixed. The magnitude of release from *P. versipora* zooxanthellae was positively correlated with the protein content of the host extract (Fig. 2, *P. versipora*, $r^2 = 0.54$, $P < 0.05$). For *Z. robustus* and *Pa. ianthina*, zooxanthellae in host extracts released only a small proportion (less than 10%) of fixed ^{14}C-carbon. The proportion released was significantly ($P < 0.05$) but only slightly higher than that released in seawater, even at concentrations of host extract (as determined by protein concentration, Fig. 2) that caused in excess of 30% release in experiments with *P. versipora*. For *C. gaboensis*, between 60 and 90% of the organic ^{14}C fixed during 1 h in host extract was subsequently found outside the cells. However, as noted previously, this host extract caused lysis of zooxanthellae and inhibited photosynthesis.

The proportion of fixed ^{14}C-carbon released from *P. versipora* and *Pa. ianthina* zooxanthellae in seawater or host extracts was monitored over 4 h of incubation (Fig. 3), and did not change significantly during that period.

To investigate the possible similarities of host factors

Table II

Photosynthetic rates in, and percentage translocation of fixed ^{14}C-products from, isolated zooxanthellae in seawater and host extract[1]

a) Photosynthetic rate (mg C · mg chl^{-1} · h^{-1})

Host	Incubation medium		
	Host extract	Seawater, light	Seawater, dark
Zoanthus robustus	0.438 ± 0.231 (7)	0.503 ± 0.287 (7)	0.018 ± 0.009 (4)
Pteraeolidia ianthina	0.578 ± 0.301 (19)	0.520 ± 0.298 (19)	0.023 ± 0.003 (6)
Capnella gaboensis	0.002 (4)	0.886 ± 0.625 (3)	n.d.
Plesiastrea versipora	0.919 ± 0.612 (12)	0.877 ± 0.594 (12)	0.018 ± 0.001 (5)

b) Percentage of fixed ^{14}C-products translocated

Host	Incubation medium	
	Host extract	Seawater, light
Zoanthus robustus	7.88 ± 1.32 (7)	2.97 ± 0.88 (7)
Pteraeolidia ianthina	6.31 ± 3.15 (19)	2.42 ± 0.34 (19)
Capnella gaboensis	70.11 ± 15.40 (4)	3.45 ± 0.92 (4)
Plesiastrea versipora	26.76 ± 15.85 (12)	4.83 ± 0.51 (12)

[1] Mean ± 95% confidence interval; number of experiments conducted for each host is shown in brackets; 10^6 zooxanthellae · ml^{-1} incubation medium for each treatment; incubation time—1 h.

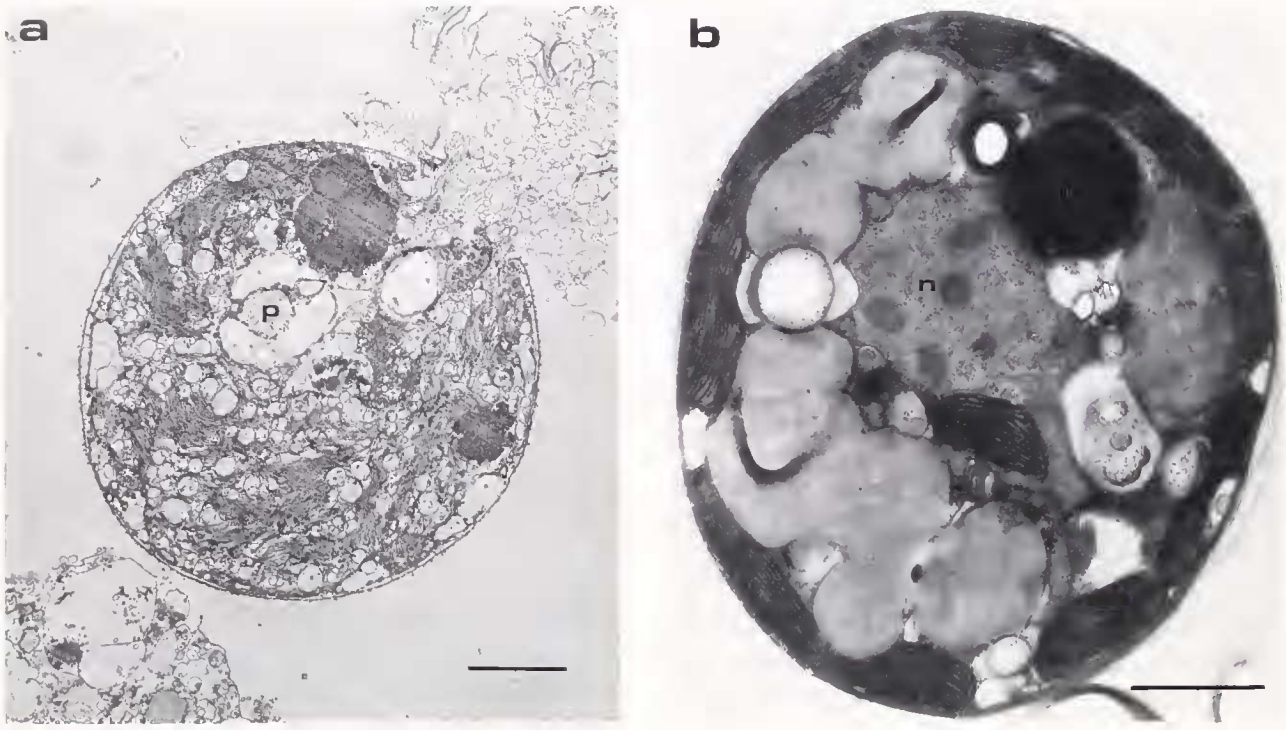

Figure 1. Ultrastructure of zooxanthellae: (a) Isolated from *Capnella gaboensis* and incubated for one hour in host extract. Note lysis and internal cellular disruption. (b) Isolated from *Plesiastrea versipora* and incubated for one hour in host extract. Transmission electron micrographs; scale bar is equivalent to 2 microns; nucleus (n), pyrenoid (p).

from different invertebrates, zooxanthellae were incubated in extracts of their own host and in extracts of hosts other than their own (Table III). *Z. robustus* and *Pa. ianthina* extracts had little effect (approximately 8% and 4–8%, respectively) on the release of ^{14}C products from both their own and other zooxanthellae. In contrast, *P. versipora* extract had no effect on *Pa. ianthina* zooxanthellae (approximately 3% release), but had a marked effect on *Z. robustus* zooxanthellae (approximately 30% release). In this latter case, the magnitude of release from *Z. robustus* zooxanthellae in host extract of *P. versipora* was about 12 times greater than in seawater, more than three times than that in *Z. robustus* extract of similar concentration, and the same as that of release by zooxanthellae from *P. versipora* in their own host extract ($P < 0.01$).

Labeled compounds detected in host extract incubations

Zooxanthellae incubated in host extract for 1 h incorporated less ^{14}C into lipid, and substantially more into neutral and organic acid fractions than they did in seawa-

Table III

Percentage of fixed ^{14}C-products released from zooxanthellae in host extract and extracts of non-host invertebrates[1]

Source of zooxanthellae	Source of host extract			
	Plesiastrea versipora (2.60)[2]	*Zoanthus robustus* (2.75)	*Pteracolidia ianthina* (2.30)	Seawater
P. versipora	27.18 ± 7.11	8.61 ± 1.00	6.49 ± 1.19	4.01 ± 0.62
Z. robustus	30.80 ± 5.62	8.52 ± 0.75	11.81 ± 3.52	2.61 ± 0.85
P. ianthina	3.97 ± 3.11	8.11 ± 4.02	7.28 ± 3.37	2.57 ± 0.41

[1] Mean ± 95% confidence interval; n = 3.

[2] Numbers in brackets refer to host extract protein concentration in mg(N)·ml^{-1}.

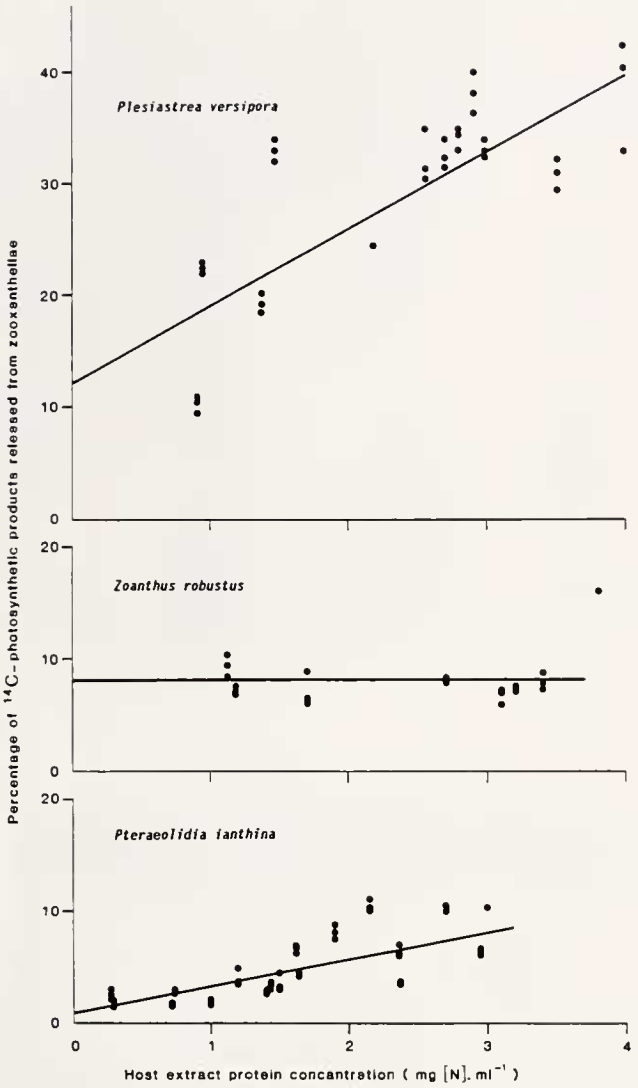

Figure 2. Release of photosynthetically fixed ^{14}C-organic carbon from zooxanthellae in host extract as a function of host extract protein content. *Plesiastrea versipora*, $r^2 = 0.54$, significant at $P < 0.01$, $y = 6.9(\times) + 12.1$. *Pteraeolidia ianthina*, $r^2 = 0.70$, significant at $P < 0.05$, $y = 2.36(\times) - 0.76$.

ter (Table IV). Neutral compounds, and to a lesser extent organic acids, were the dominant soluble labeled compounds detected outside the zooxanthellae in both seawater and host extract treatments. About 30% of the extracellular label in each treatment was in the form of glycerol (Table V), representing about 10% and 2% of the total ^{14}C fixed by zooxanthellae in host extract and seawater, respectively. There were 3–5 unidentified compounds detected extracellularly in each treatment, representing about 10% and 0.3% of the total ^{14}C fixed by zooxanthellae in host extract and seawater, respectively. In addition to neutral compounds, labeled glycollate, pyruvate, malate, and leucine were detected extracellularly in host extract but not seawater. Labeled fructose and aspartate were detected extracellularly in seawater but not host extract. Labeled alanine was found in both treatments, but was not the major labeled amino acid detailed extracellularly in either case. The major amino acid present was not identified.

Effect of P. versipora *host extract on pre-formed products of photosynthesis*

Zooxanthellae incorporating ^{14}C into photosynthetic products during an initial treatment of incubation for 1 h in seawater with label, retained most of those ("preformed") labeled products when subsequently incubated in host extract for 1 h. In contrast, zooxanthellae having an initial treatment in seawater without label but which incorporated ^{14}C into photosynthetic products during subsequent incubation for 1 h in host extract, released a significant proportion of labeled products formed during that incubation (Table VI).

Effect of boiling on host extract activity

Heating of *P. versipora* extract at 100°C for 10 min resulted in a significant loss of release-inducing activity (Table VIIa). However, zooxanthellae incubated in

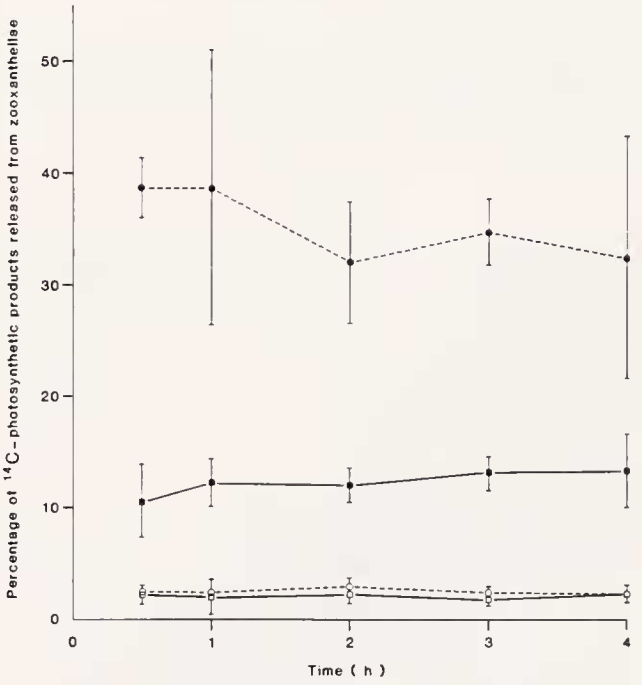

Figure 3. Release of photosynthetically fixed ^{14}C-organic carbon by zooxanthellae with time during extended exposure (4 h) to host extract. Zooxanthellae isolated from *Plesiastrea versipora* (○) and *Pteraeolidia ianthina* ([]) and incubated in seawater; zooxanthellae isolated from *P. versipora* (●) and *Pa. ianthina* (■) and incubated in host extract.

Table IV

Incorporation of ^{14}C into zooxanthellae lipid, amino acid, organic acid, and neutral compound classes, and percentage of each class detected extracellularly after incubation for one hour in seawater or Plesiastrea versipora *extract*

#	Class of compound	Zooxanthellae in seawater			Zooxanthellae in host extract		
		Intracellular	Extracellular[1]	Total	Intracellular	Extracellular[1]	Total
1	Lipid	75.4	0.2	75.6	22.5	0.5	23.0
	Amino acid	8.5	0.5	9.0	11.7	1.1	12.8
	Organic acid	3.9	0.1	4.0	11.7	9.6	21.3
	TOTAL	96.0	4.0	100.0	67.9	32.1	100.0
2	Lipid	54.1	0.6	54.7	9.0	5.3	14.3
	Amino acid	11.4	0.4	11.8	13.1	2.3	15.4
	Organic acid	9.1	1.0	10.1	13.5	18.6	32.1
	Neutral	20.5	2.9	23.4	27.0	11.2	38.2
	TOTAL	95.1	4.9	100.0	62.6	37.4	100.0
3	Lipid	53.3	0.8	54.1	24.7	0.4	25.1
	Amino acid	10.9	0.5	11.4	12.9	0.8	13.7
	Organic acid	9.1	1.3	10.4	12.8	7.7	20.5
	Neutral	20.4	3.7	24.1	24.2	16.5	40.0
	TOTAL	93.7	6.3	100.0	74.6	25.4	100.0

[#] Experiment number.

[1] "Total" extracellular for each experiment = percent ^{14}C released from zooxanthellae.

heated host extract still released slightly more ^{14}C-products than did cells incubated in seawater.

Effect of dialysis on host extract activity

The release-inducing activity of *P. versipora* extract did not decrease when the extract was dialyzed against seawater for 6 h at 4°C (Table VIIb).

Discussion

In this study, aspects of the interaction between four temperate marine invertebrates and their symbiotic zooxanthellae were examined. Extracts of the stony coral *P. versipora* may contain "host factors" that control translocation of photosynthetic products from symbiont to host. In the other invertebrates examined, no evidence of a "host factor" effect on translocation of photosynthates was found. Therefore, these observations extend to temperate symbioses the likelihood that host factors are not a universal property of symbiotic associations involving zooxanthellae. We also found that host extracts may affect zooxanthellae in previously unreported ways, apparently through their effect on metabolic pathways. Thus, in at least some symbioses, the host may have a much greater control over zooxanthellar processes then previously thought.

The transfer of fixed carbon *in vivo* from symbiont to animal tissues is significant in the nutrition of the host and has been investigated for a number of zooxanthellae/invertebrate symbioses. In tropical invertebrates, zooxanthellae release between 24 and 27% of total fixed carbon to the host in sea anemones (von Holt and von Holt, 1968a), 26–55% in corals (Muscatine and Cernichiari, 1969; Muscatine *et al.*, 1984), and 20–42% in zoanthids (von Holt and von Holt, 1968b; Trench, 1971a). Studies of translocation in temperate invertebrates are few, although significant translocation has been reported for the two temperate sea anemones, *Anthopleura elegantissima* (56%, Trench, 1971a) and *Anemonia sulcata* (60%, Taylor, 1969), respectively.

One objective of the present study, therefore, was to determine whether there was evidence for translocation of photosynthetic products from zooxanthellae to host in diverse temperate invertebrates. Results showed that zooxanthellae *in vivo* photosynthesized at rates comparable to those reported for zooxanthellae in tropical invertebrates (Porter, 1976; Scott and Jitts, 1977; Muscatine *et al.*, 1984), and that a variable but significant proportion of total fixed carbon was subsequently transferred to the host tissues. Thus, the proportion translocated ranged from 11.8% to 35.2% for *Z. robustus*, 23.8% to 47.5% for *Pa. ianthina*, and 18.0% to 19.1% for *C. gaboensis*. Difficulties encountered in achieving complete separation of tissues from the calcareous skeleton of *P. versipora* prevented any statement being made concerning photosynthesis and translocation in this association when it is intact. The possibility of seasonal variation in the translocation of fixed carbon to the host

Table V

Percentage of total extracellular fixed ^{14}C present as specific compounds after incubation of zooxanthellae for one hour in seawater or Plesiastrea versipora *extract*[1]

Compounds	Percentage of total ^{14}C released into seawater (%)	Percentage of total ^{14}C released into host extract (%)
LIPID	12.7	1.1
AMINO ACIDS		
Alanine	3.4	0.1
Aspartate	0.4	–
Leucine	–	2.3
Unidentified	4.1	1.0
ORGANIC ACIDS		
Lactate	2.1	8.5
Oxaloacetate	6.1	–
Pyruvate	–	2.9
Glycollate	–	5.4
Malate	–	3.8
Unidentified	12.4	9.7
NEUTRAL		
Glycerol	33.5	31.5
Glucose	14.4	1.7
Fructose	6.1	–
Unidentified	4.8	32.2
TOTAL	100.0	100.0

[1] % recovery of ^{14}C from incubation medium was 85% and 95% for seawater and host extract, respectively.

was suggested for *Z. robustus*, but was not demonstrated conclusively. Therefore, if the symbioses examined in this study are representative of temperate interactions involving zooxanthellae, it is apparent that they are similar to their tropical counterparts in terms of short-term movement of substantial amounts of fixed carbon from symbiont to host. The contribution of symbionts to the energy demands of the hosts in this study and in temperate invertebrates in general remains to be elucidated.

This study, and previous ones addressing the question of ways in which the host may exert control over zooxanthellae in symbiosis, relied on *in vitro* experimentation using host extracts and isolated zooxanthellae. Interpretation of results in studies of this nature is made difficult due to the possibility of experimental artifacts as a consequence of extraction or incubation procedures. Lytic effects of host extracts on isolated zooxanthellae have been reported previously (Steele and Goreau, 1977), and, in the present study, extracts of *C. gaboensis* caused both lysis of zooxanthellae and inhibition of photosynthesis. However, for *P. versipora, Pa. ianthina,* and *Z. robustus,* the microscopic and biochemical evidence suggests that isolation from these hosts and subsequent *in vitro* experimentation in host extracts had no detrimental effects on zooxanthellae. First, the photosynthetic rates of isolated zooxanthellae were equal to or higher than rates measured for zooxanthellae in the intact association, and were comparable to rates determined for isolated zooxanthellae from other marine invertebrates (Burris, 1977; Dunstan, 1982; Muller-Parker, 1984). Second, zooxanthellae in seawater retained more than 95% of the organic carbon fixed during four hours of incubation. Third, following incubation in host extract or seawater, zooxanthellae were microscopically indistinguishable from zooxanthellae *in vivo*.

Many studies, principally of tropical invertebrates, have suggested that the host may exert control over its symbiotic partner in at least two ways, namely by affecting the photosynthetic rate in zooxanthellae and by stimulating release of photosynthetic products. A second objective of this study was to seek evidence for similar host control of these processes in temperate invertebrates, and to determine whether there was evidence for host control of other zooxanthellar processes.

Table VI

Effect of Plesiastrea versipora *extract on release of pre-formed ^{14}C-photosynthetic products from zooxanthellae*

Initial treatment	Pre-formed ^{14}C-products present	Subsequent treatment	% Release of ^{14}C-products during 1 h in "subsequent treatment" Experiment 1	Experiment 2
SW	–	SW + ^{14}C	5.54 ± 2.83	5.95 ± 2.19
	–	HE + ^{14}C	22.43 ± 2.97	32.19 ± 2.12
SW + ^{14}C	+	SW	10.68 ± 2.71	4.60 ± 0.95
	+	HE	9.73 ± 2.53	3.92 ± 0.81

[1] Zooxanthellae (10^6/ml) incubated for 1 h in the listed treatments. SW = seawater; HE = host extract; $^{14}C = NaH^{14}CO_3$, 5 $\mu Ci \cdot ml^{-1}$.
[2] Zooxanthellae removed from "initial treatment" and incubated for 1 h in the listed "subsequent treatments."
[3] Mean ± 95% confidence interval, n = 3.

Table VII

Effect of 100°C and dialysis on ^{14}C release-inducing activity of Plesiastrea versipora *host extract*[1]

a) 100°C

	% Release of ^{14}C-products from zooxanthellae	
	Experiment 1	Experiment 2
Seawater	4.67 ± 1.14	4.70 ± 0.95
Host extract	30.37 ± 1.13	47.68 ± 1.13
100°C-treated host extract[2]	8.53 ± 1.11	12.68 ± 1.15

b) Dialysis

	% Release of ^{14}C-products from zooxanthellae
	Experiment 1
Seawater	6.40 ± 1.94
Fresh host extract	28.50 ± 3.78
Dialyzed (6 h, 4°C) extract[3]	37.40 ± 3.21
Undialyzed (6 h, 4°C) extract[3]	36.50 ± 1.59

[1] Mean ± 95% confidence interval; n = 3.

[2] Fresh host extract treated at 100°C for 10 min.

[3] Extract dialyzed at 4°C for 6 h against 2 changes of phosphate buffer, or held at 4°C for 6 h (undialyzed extract).

No evidence was found that any of the host invertebrates in this study stimulated enhanced photosynthetic rates in their symbionts. Zooxanthellae in the intact association had rates the same as, or lower than, those freshly isolated, and there was no difference in rates *in vitro* for isolated zooxanthellae in host extract or seawater. This result contrasts with two previous ones, the first for zooxanthellae from *A. elegantissima*, which were reported to fix ^{14}C in host extract at rates an order of magnitude higher than in seawater (Trench, 1971c), and the second by Muscatine *et al.* (1972) who observed an opposite trend in experiments with the hydrozoan *Millepora alcicornis*. One explanation for the different results in these three studies may be found in the experimental procedures involved. In the present study, the pH of host extracts was measured and adjusted to that of seawater at the beginning of each experiment. This may be significant, particularly considering the recent demonstration of a correlation between increased photosynthetic rate in zooxanthellae and high incubation pH (Hoegh-Guldberg, unpub. data). In the other studies noted above, the pH of the host extract was not reported, but it may have been sufficiently different from seawater controls to account for the observed differences in photosynthetic rates. A second explanation is that hosts' ability to influence photosynthetic rates in their symbiotic partners varies, and that the results of this and previous studies reflect that variability.

The second way in which hosts may exert control over zooxanthellae is through stimulation of release of photosynthetic products. Evidence that host tissues contain factors controlling release in invertebrates having zooxanthellae has been found in one temperate anemone [*A. elegantissima* (Trench, 1971c)] and in several tropical corals (Muscatine, 1967; Muscatine *et al.*, 1972). Only for *P. versipora* was strong evidence found for the presence of host release factors in this study. Relative to seawater controls, host extract of *P. versipora* stimulated release of a large proportion (up to 42%) of the carbon fixed during photosynthesis in that extract. The rate of release remained constant over four hours of incubation, and the magnitude of release was correlated with the total protein concentration of the extract. It is likely that the constant rate of release reflects the stability of the host extract factor during the incubation period. Unpublished data suggests that removal of zooxanthellae from host extract and subsequent incubation in seawater with ^{14}C causes an immediate reversion to levels of release characteristic of those in seawater. Therefore, host factor appears to cause only a temporary (during exposure) effect on release patterns.

Preliminary attempts to chemically characterize an active component from the host extract of *P. versipora* demonstrated that the release-inducing activity did not pass through dialysis tubing (nominal pore size 10,000 D) and was almost completely destroyed (approximately 80% reduction) by heating. The correlation of the magnitude of release with protein concentration, constant rate of release of fixed products, sensitivity to heat, and non-dialyzability are consistent with the host extract containing an active chemical constituent, possibly proteinaceous in nature and stimulating release of photosynthetic products from zooxanthellae. It is possible that "host factor" activity in this and previous studies is an artifact of the experimental procedure. However, the identification of a chemical having release-inducing activity *in vitro* will be a first major step in assessing the role of such chemicals in intact associations.

Results of experiments with *P. versipora* suggested the possibility of a third, and previously unreported, level of control by the host over zooxanthellar processes. Extracts of this host had a marked effect on the metabolism of zooxanthellae, resulting principally in the incorporation of photosynthetically fixed carbon into glycerol and other neutral compounds (predominantly monosaccharides). This contrasted with the incorporation of most of the photosynthetically fixed carbon into lipids in cells incubated in seawater. This is particularly interesting in view of the observation that only carbon fixed in the presence of host extract was released, while photosyn-

thetic products formed prior to exposure to extract were retained during incubation in extract. This latter result suggests that pre-formed products have entered pathways or pools where they are not affected by host extracts. Therefore, it may be that these results demonstrate a degree of host control over metabolic processes in the symbiont, whereby organic carbon, which is normally incorporated into lipids in free-living dinoflagellates, is diverted in symbiosis to pathways that result in the formation of products that may be more readily translocated to the host.

Glycerol constituted a large proportion of the algal photosynthetic products detected outside zooxanthellae after incubation in *P. versipora* extracts. It was not possible, using available methods, to demonstrate conclusively that the glycerol, or other labeled compounds detected extracellularly, was translocated, and not formed in the incubation medium as a result of heterotrophic fixation or the activity of host enzymes on unidentified compounds released from zooxanthellae. However, non-zooxanthellae (heterotrophic) fixation in host extracts of other marine invertebrates is negligible (Trench, 1971b), and glycerol is thought to be the major product translocated from zooxanthellae in marine invertebrates (Trench, 1979; Battey and Patton, 1984); up to 90% of the organic carbon translocated in short-term incubations has been reported to be in this form. Other compounds translocated include lipids (Patton *et al.*, 1977a; Kellog and Patton, 1983; Battey and Patton, 1984) and amino acids (Trench, 1971b, c). Glycerol is a lipid-soluble substance that moves easily through biological membranes (Davson and Danielli, 1952; Dainty and Ginzburg, 1964; Wright and Diamond, 1969). If the translocation of glycerol is not restricted by a membrane barrier, it seems unlikely that host factors act by affecting membrane permeability to glycerol at the interface between host and symbiont, as has been suggested (Trench, 1979) in previous studies. An alternative possibility is that host factors operate by influencing glycerol catabolism and anabolism, thereby determining the concentration gradient of glycerol between host and symbiont.

Experiments with the other invertebrates used in our study provided no strong evidence for the presence of host factors influencing translocation of photosynthetic products from zooxanthellae. This raises the question of whether temperate invertebrates in general possess host factor activity. *C. gaboensis* extract inhibited photosynthesis and lysed freshly isolated zooxanthellae. This result clearly demonstrates the possibility of artifacts in experiments of this nature, and points to the need for assessment of algal condition and physiology to detect such artifacts. Extracts prepared from *Z. robustus* and *Pa. ianthina* stimulated little release of carbon from their zooxanthellae. These latter results are in contrast with the results obtained for *P. versipora* and with the substantial release detected for *Z. robustus* and *Pa. ianthina* in the intact association. One explanation is that the active constituent or a required cofactor is destroyed during the isolation procedure for *Z. robustus* and *Pa. ianthina*. Indirect evidence that this may be the case for *Z. robustus* comes from the observation that zooxanthellae from this host released a significant proportion of photosynthetic products when exposed to *P. versipora* extract. This observation would also suggest a similarity in host factors in these two invertebrates, a phenomenon previously reported for two tropical invertebrates (Muscatine, 1967). A second explanation is that host factors are absent in *Z. robustus* and *Pa. ianthina*. Given the diversity of invertebrate hosts and different species of zooxanthellae (Blank and Trench, 1985), it is quite possible that nutrient exchange in some symbioses may be mediated, not through the activity of host factors, but through mechanisms that remain to be elucidated.

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